SYNTHESIS OF HIPPURIC ACID IN LIVER HOMOGENATE*

Sirs:

The synthesis of hippuric acid from benzoic acid and glycine resembles in several respects the synthesis of a peptide bond. A CONH group is formed, it is α to a carboxyl group, and the free energy of its formation is of the same order of magnitude. The synthesis was demonstrated in liver and kidney slices of a number of animals; it is inhibited by 0.001 M KCN, which is in accord with the view that the necessary free energy is derived from an oxidation.[1]

It has not been possible with tissue slices to elucidate further the energy-coupling reaction. The cell walls evidently retarded or blocked entry into the cells of possible free energy donors. Consequently we have sought experimental conditions which permit the reaction to proceed in tissue extracts in which the cells were disrupted.

We have now obtained synthesis of hippuric acid from benzoic acid and glycine in homogenized guinea pig liver suspended in a phosphate-saline solution. To obtain more than traces of synthesis it was necessary to homogenize the liver in the presence of the substrate, *i.e.* of benzoic acid and glycine. The yield of hippuric acid was nearly doubled when adenylic acid and α-ketoglutaric acid were added. Evidently the oxidation of the latter substance provided ATP[2] which in turn furnished the free energy for the synthesis. This surmise is based on experiments on the methylation of guanidoacetic acid by methionine, a reaction which requires ATP.[3]

The reaction mixture contained in 500 ml. 10 gm. of homogenized guinea pig liver, 0.02 M glycine, 0.01 M benzoic acid, 0.001 M adenylic acid, and 0.01 M α-ketoglutaric acid in a phosphate-saline solution.[4] The reaction was carried out under oxygen at 38°. The same results were obtained at pH 7.0 and 7.5. Hippuric acid was determined by isolation and identified by crystal form, melting point, and elementary analysis.

Some characteristic findings appear in the table.

Thermodynamic data give the equilibrium constant for the hydrolysis of hippuric acid under our experimental conditions as 211;[1] accordingly the amount of hippuric acid to be expected from 0.02 M glycine and 0.01 M benzoic acid by mass action alone is 0.026 mg. The isolation of 30 mg.

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[1] Borsook, H., and Dubnoff, J. W., *J. Biol. Chem.*, **132**, 307 (1940).

[2] Ochoa, S., *J. Biol. Chem.*, **155**, 87 (1944).

[3] These findings will be reported shortly.

[4] Cohen, P. P., and Hayano, M., *J. Biol. Chem.*, **166**, 251 (1946).

	Hippuric acid isolated
	mg.
Liver alone	0
" homogenized first and complete reaction mixture added subsequently	0–3
Liver homogenized with benzoic acid and glycine	17
" " " " " glycine, adenylic acid, and α-ketoglutaric acid, 0 time	0
Liver homogenized with benzoic acid, glycine, adenylic acid, and α-ketoglutaric acid, 4 hrs. incubation	30

indicates that the synthesis had occurred through coupling with an energy-yielding reaction, presumably ATP. Under our experimental conditions the isolation is not quantitative; at least 10 mg. are not recovered.

Experiments on the methylation of guanidoacetic acid indicated that guinea pig liver homogenate provides some ATP. This probably accounts for the yield of 17 mg. of hippuric acid when no adenylic acid and α-ketoglutaric acid were added to the reaction mixture.

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